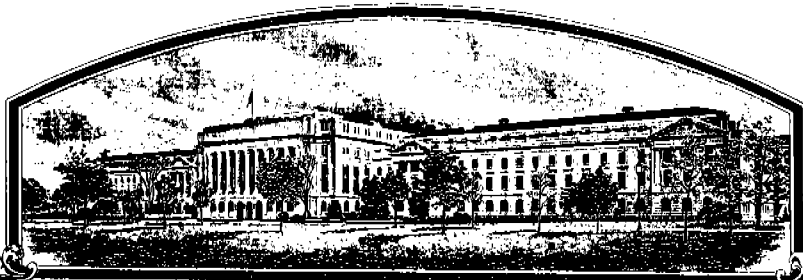


No.



7600063

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

Asgrow Seed Company

Whereas, THERE HAS BEEN PRESENTED TO THE
Secretary of Agriculture

AN APPLICATION REQUESTING A CERTIFICATE OF PROTECTION FOR AN ALLEGED NOVEL VARIETY OF SEXUALLY REPRODUCED PLANT, THE NAME AND DESCRIPTION OF WHICH ARE CONTAINED IN THE APPLICATION AND EXHIBITS, A COPY OF WHICH IS HEREUNTO ANNEXED AND MADE A PART HEREOF, AND THE VARIOUS REQUIREMENTS OF LAW IN SUCH CASES MADE AND PROVIDED HAVE BEEN COMPLIED WITH, AND THE TITLE THERETO IS, FROM THE RECORDS OF THE PLANT VARIETY PROTECTION OFFICE, IN THE APPLICANT(S) INDICATED IN THE SAID COPY, AND WHEREAS, UPON DUE EXAMINATION MADE, THE SAID APPLICANT(S) IS (ARE) ADJUDGED TO BE ENTITLED TO A CERTIFICATE OF PLANT VARIETY PROTECTION UNDER THE LAW.

NOW, THEREFORE, THIS CERTIFICATE OF PLANT VARIETY PROTECTION IS TO GRANT UNTO THE SAID APPLICANT(S) AND THE SUCCESSORS, HEIRS OR ASSIGNS OF THE SAID APPLICANT(S) FOR THE TERM OF *seventeen* YEARS FROM THE DATE OF THIS GRANT, SUBJECT TO THE PAYMENT OF THE REQUIRED FEES AND PERIODIC REPLENISHMENT OF VIABLE BASIC SEED OF THE VARIETY IN A PUBLIC REPOSITORY AS PROVIDED BY LAW, THE RIGHT TO EXCLUDE OTHERS FROM SELLING THE VARIETY, OR OFFERING IT FOR SALE, OR REPRODUCING IT, OR IMPORTING IT, OR EXPORTING IT, OR USING IT IN PRODUCING A HYBRID OR DIFFERENT VARIETY THEREFROM, TO THE EXTENT PROVIDED BY THE PLANT VARIETY PROTECTION ACT STAT. 1542, AS AMENDED, 7 U.S.C. 2321 ET SEQ.)

SOYBEAN

'A3001'

In Testimony Whereof, I have hereunto set
my hand and caused the seal of the Plant
Variety Protection Office to be affixed
at the City of Washington
this 18th day of November in
the year of our Lord one thousand nine
hundred and seventy-six

Attest:

R. J. Rollin

Commissioner
Plant Variety Protection Office
Grain Division
Agricultural Marketing Service

John G. T. July
Secretary of Agriculture

APPLICATION FOR PLANT VARIETY PROTECTION CERTIFICATE

INSTRUCTIONS: See Reverse.

1. VARIETY NAME OR TEMPORARY DESIGNATION A 3001	2. KIND NAME Soybean	FOR OFFICIAL USE ONLY PV NUMBER 7600063	
3. GENUS AND SPECIES NAME Glycine Max	4. FAMILY NAME (Botanical) Leguminosae	FILING DATE 3-30-76	TIME 1 P.M.
	5. DATE OF DETERMINATION 1972	FEE RECEIVED \$ 250.00	BALANCE DUE \$ —
		\$ 250.00	\$ —
6. NAME OF APPLICANT(S) Asgrow Seed Company	7. ADDRESS (Street and No. or R.F.D. No., City, State, and ZIP Code) Kalamazoo, MI 49001	8. TELEPHONE AREA CODE AND NUMBER (616) 385-6605	
9. IF THE NAMED APPLICANT IS NOT A PERSON, FORM OF ORGANIZATION: (Corporation, partnership, association, etc.) Corporation		10. STATE OF INCORPORATION Delaware	11. DATE OF INCORPORATION 3/22/68

12. Name and mailing address of applicant representative(s), if any, to serve in this application and receive all papers:

John A. Batcha
Asgrow Seed Company
Unit 9630-190-1
Kalamazoo, MI 49001

13. CHECK BOX BELOW FOR EACH ATTACHMENT SUBMITTED:

- ☒ 13A. Exhibit A, Origin and Breeding History of the Variety (See Section 52 of the Plant Variety Protection Act.)
- ☒ 13B. Exhibit B, Botanical Description of the Variety
- ☒ 13C. Exhibit C, Objective Description of the Variety
- ☒ 13D. Exhibit D, Data Indicative of Novelty
- ☒ 13E. Exhibit E, Statement of the Basis of Applicant's Ownership

14A. Does the applicant(s) specify that seed of this variety be sold by variety name only as a class of certified seed? (See Section 83(a). (If "Yes," answer 14B and 14C below.) ☐ YES ☒ NO14B. Does the applicant(s) specify that this variety be limited as to number of generations? ☐ YES ☐ NO14C. If "Yes," to 14B, how many generations of production beyond breeder seed? ☐ FOUNDATION ☐ REGISTERED ☐ CERTIFIED

The applicant declares that a viable sample of basic seed of this variety will be deposited upon request before issuance of a certificate and will be replenished periodically in accordance with such regulations as may be applicable.

The undersigned applicant(s) of this sexually-reproduced novel plant variety believes that the variety is distinct, uniform, and stable as required in Section 41 and is entitled to protection under the provisions of Section 42 of the Plant Variety Protection Act.

Applicant is informed that false representation herein can jeopardize protection and result in penalties.

March 23, 1976
(DATE)

John A. Batcha
(SIGNATURE OF APPLICANT)

(DATE)

(SIGNATURE OF APPLICANT)

INSTRUCTIONS

GENERAL: Send an original copy of the application, exhibits and \$250.00 fee to U.S. Dept. of Agriculture, Agricultural Marketing Service, Grain Division, 6525 Belcrest Road, Hyattsville, Maryland 20782. (See Section 180.175 of the regulations and rules of practice.) Retain one copy for your files. All items on the face of the form are self-explanatory unless noted below.

ITEM

- 5 Insert the date the applicant determined that he had a new variety based on the definition in Section 41 (a) of the Act and decision is made to increase the seed.
- 13a First, give the genealogy, including public and commercial varieties, lines, or clones used, and the breeding method. Second, give the details of subsequent stages of selection and multiplication. Third, indicate the type and frequency of variants during reproduction and multiplication and state how these variants may be identified. Fourth, provide evidence on stability.
- 13b First, give any special characteristics of the seed and of the plant as it passes through the seedling stage, flowering stage and the fruiting stage. Second, describe the mature plant and compare it with a similar commercial variety grown under the same conditions, and indicate the differences.
- 13c A supplemental form will be furnished by the PVPO to describe in detail a variety for each kind of seed.
- 13d Provide complete data indicative of novelty. Seed and plant specimens or photographs of seed and plant comparisons clearly indicating novelty may be submitted. Seeds submitted may be sterile.
- 13e Indicate whether applicant is the actual breeder, the employer of the breeder, the owner through purchase or inheritance, etc.

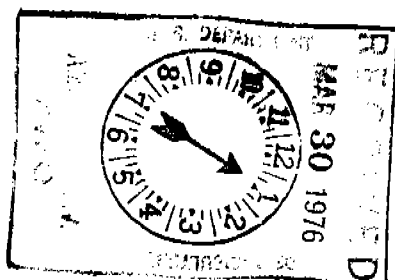


EXHIBIT A

ORIGIN AND BREEDING HISTORY OF A3001

Original Cross: Beeson X Calland

Made in Summer of 1968 in Iowa

1968-69	F1 and F2 generations grown in winter nursery in Hawaii
1969	F3 bulk from F2 pod picks grown in Iowa
1969-70	F4 bulk from F3 pod picks grown in winter nursery in Puerto Rico. Single plant selections made and individually threshed.
1970	F5 plant rows grown in Iowa. Plant row 70-8409 was bulk harvested and eventually became A3001.
1971	Preliminary yield tests grown at two locations in Iowa.
1972	Advanced yield tests in Iowa, Illinois, and Indiana.
1973	Advanced yield tests were conducted in Iowa, Illinois, Ohio, and Indiana. Approximately 600 bushels of seed stock was produced. This seed had white flowered and tall off types. The % of off types was approximately 5%.
1974	Advanced Yield Tests, State Tests and Demonstration Plots. 30 acres were rogued twice and this purified seed lot was designated A3001.
1975	Seed stock of A3001 was produced. Testing continued in Asgrow and State Trials. A3001 has proved stable in growouts in 1975 and in Florida in the Winter of 1975-76.

March 23, 1976



Asgrow Seed Company
subsidiary of The Upjohn Company

Kalamazoo, Michigan 49001

20 July 1976

PV # 7600063

EXHIBIT ADDENDUM - EXHIBIT A

Mr. Robert J. Snyder, Examiner
Plant Variety Protection Office
Grain Division
U.S. Department of Agriculture
National Agricultural Library
Beltsville, Maryland 20705

Dear Bob:

The tall and white flowered offtypes appearing in our 1973 crop production of A3001 soybean application number 7600063 have been rogued and eliminated. The variety A3001 is now uniform and stable based on our observations.

Very truly yours,

John A. Batcha, Manager
Inventory and Distribution

JAB/ghs

EXHIBIT B

Bontanical Description of A3001 Soybean

A3001 is a group III maturity soybean cultivar highly adapted to all those soybean producing areas where cultivars, such as Wayne and Calland, are grown.

Its average maturity of A3001 is 1975 tests was two days later than Wayne and two days earlier than Calland.

The plants of A3001 are tall, of bushy type growth and of an indeterminate plant habit. The leaf shape and size are similar to those of its parent cultivar, Calland. The leaves are medium in size, medium green in color and covered with a normal type of brown pubescence. Pods are filled with mostly three-seeded pods with some two-seeded ones also occurring.

Seeds of A3001 are medium in size averaging about 2600 seeds per pound. The seeds have a black hilum and a medium shiny seed coat luster.

A3001 has been tested extensively for resistance to Phytophthora root rot in greenhouse and field tests. In all tests with Races 1 and 2 of Phytophthora, A3001 has expressed the resistant reaction.

A3001 has poor hypocotyl elongation under constant 25° C temperature. A3001 rating in this hypocotyl elongation test is five as compared to one for Calland.

A3001 has an intermediate reaction to iron chlorosis conditions found on soils of high pH (over 7.5).

March 23, 1976

OBJECTIVE DESCRIPTION OF VARIETY
SOYBEAN (GLYCINE MAX)

INSTRUCTIONS: See Reverse.

NAME OF APPLICANT(S) Asgrow Seed Company	FOR OFFICIAL USE ONLY
ADDRESS (Street and No., or R.F.D. No., City, State, and ZIP Code) Kalamazoo, MI 49001	PVPO NUMBER 7600063
	VARIETY NAME OR TEMPORARY DESIGNATION A 3001

Place the appropriate number that describes the varietal character of this variety in the boxes below.

1. SEED SHAPE: <input type="text" value="1"/> 1 = SPHERICAL 2 = SPHERICAL FLATTENED 3 = ELONGATE 4 = OTHER (Specify)	
2. SEED COAT COLOR: SHADE: <input type="text" value="1"/> 1 = YELLOW 2 = GREEN 3 = BROWN 4 = BLACK <input type="text" value="2"/> 1 = LIGHT 2 = MEDIUM 3 = DARK 5 = OTHER (Specify)	
3. SEED COAT LUSTER: <input type="text" value="2"/> 1 = DULL 2 = SHINY	4. SEED SIZE <input type="text" value="1"/> <input type="text" value="7"/> GRAMS PER 100 SEEDS
5. HILUM COLOR: SHADE: <input type="text" value="6"/> 1 = BUFF 2 = YELLOW 3 = BROWN 4 = GRAY 5 = IMPERFECT BLACK <input type="text" value="3"/> 1 = LIGHT 2 = MEDIUM 3 = DARK 6 = BLACK 7 = OTHER (Specify)	
6. COTYLEDON COLOR: <input type="text" value="2"/> 1 = YELLOW 2 = GREEN	7. LEAFLET SIZE (See Reverse): <input type="text" value="2"/> 1 = SMALL 2 = MEDIUM 3 = LARGE
8. LEAFLET SHAPE: <input type="text" value="2"/> 1 = OVATE 2 = OBLONG 3 = LANCEOLATE 4 = ELLIPTICAL 5 = OTHER (Specify)	
9. LEAF COLOR (See reverse): <input type="text" value="2"/> 1 = LIGHT GREEN 2 = MEDIUM GREEN 3 = DARK GREEN	10. FLOWER COLOR: <input type="text" value="2"/> 1 = WHITE 2 = PURPLE 3 = OTHER (Specify)
11. POD COLOR: <input type="text" value="2"/> 1 = TAN 2 = BROWN 3 = BLACK	12. POD SET: <input type="text" value="1"/> 1 = SCATTERED 2 = CONCENTRATED
13. PLANT PUBESCENCE COLOR: SHADE: <input type="text" value="2"/> 1 = GRAY 2 = BROWN 3 = OTHER (Specify) <input type="text" value="2"/> 1 = LIGHT 2 = MEDIUM 3 = DARK	
14. PLANT TYPES (See Reverse): <input type="text" value="2"/> 1 = SLENDER 2 = BUSHY 3 = INTERMEDIATE	15. PLANT HABIT: <input type="text" value="2"/> 1 = DETERMINATE 2 = INDETERMINATE 3 = OTHER (Specify)
16. HYPOCOTYL COLOR: <input type="text" value="1"/> 1 = GREEN 2 = PURPLE	17. SEED PROTEIN: <input type="text" value=""/> 1 = A 2 = B
18. NUMBER OF DAYS TO FLOWERING (Place a zero in first box (e.g. <input type="text" value="0"/> <input type="text" value="9"/>) when days are 9 or less.) <input type="text" value="7"/> <input type="text" value="2"/>	19. MATURITY GROUP: <input type="text" value="5"/> 1 = 00 2 = 0 3 = I 4 = II 5 = III 6 = IV 7 = V 8 = VI 9 = VII 10 = VIII
20. SIZE OF 10 DAY OLD SEEDLING GROWN UNDER CONSTANT LIGHT (Growth Chamber) AT 25° C. (Place a zero in first box (e.g. <input type="text" value="0"/> <input type="text" value="1"/> <input type="text" value="2"/>) when size is 9 mm. or less.) <input type="text" value="1"/> <input type="text" value="4"/> <input type="text" value="3"/> MM. LENGTH OF SEEDLING <input type="text" value="2"/> <input type="text" value="2"/> MM. LENGTH OF COTYLEDON <input type="text" value="1"/> <input type="text" value="5"/> MM. WIDTH OF COTYLEDON	
21. DISEASE: (Enter 0 = Not Tested; 1 = Susceptible; 2 = Resistant)	
<input type="text" value="1"/> BACTERIAL PUSTULE <input type="text" value="1"/> SOYBEAN CYST <input type="text" value="1"/> DOWNY MILDEW <input type="text" value="1"/> PURPLE STAIN <input type="text" value="1"/> POD AND STEM BLIGHT <input type="text" value="1"/> ROOT KNOT	
<input type="text" value="2"/> FROGEYE <input type="text" value="2"/> STEM CANKER <input type="text" value="2"/> PHYTO-PHTHORA <input type="text" value="1"/> BROWN STEM ROT <input type="text" value="1"/> TARGET SPOT <input type="text" value="2"/> BROWN SPOT	
<input type="text" value="1"/> BUD BLIGHT <input type="text" value="1"/> WILDFIRE <input type="text" value="1"/> RHIZOCTONIA ROT <input type="text" value="2"/> OTHER (Specify) Powdery Mildew <input type="text" value="2"/> Bacterial Blight	

March 23, 1976

22. INDICATE WHICH VARIETY MOST CLOSELY RESEMBLES THAT SUBMITTED.

CHARACTER	NAME OF VARIETY	CHARACTER	NAME OF VARIETY
Plant shape	Calland	Petiole angle	Calland
Leaf shape	Calland	Seed size	Calland
Leaf color	Calland	Seed shape	Calland
Leaf surface	Calland	Seedling pigmentation	Calland

23. GIVE DATA FOR SUBMITTED AND SIMILAR STANDARD VARIETY:

VARIETY	NO. OF DAYS TO MATURITY	LODGING SCORE	PLANT HEIGHT In.	LEAF SIZE		CONTENT		AVERAGE NO. OF PODS PER PLANT	IODINE NO.
				Width	Length	Protein	Oil		
Submitted	152	2.3	42	9.0	7.0		%	76	
Name of similar variety Calland	155	2.5	43	9.5	7.0			73	

INSTRUCTIONS

GENERAL: The following publications may be used as a reference aid for completing this form:

1. Scott, Walter O. and Samuel R. Aldrich, 1970, Modern Soybean Production, The Farmer Quarterly.
2. Norman, A. G., 1963, The Soybean: Genetics, Breeding, Physiology, Nutrition, Management.
3. McKie, J. W., and K. L. Anderson, 1970, The Soybean Book.

LEAF COLOR: Nickerson's or any recognized color fan may be used to determine the leaf color of the described variety. The following Soybean varieties may be used as a guide to identify the colors listed on the form.

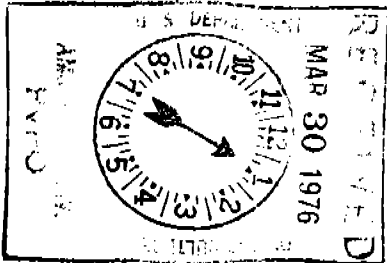
COLOR	VARIETY
Light Green	"Ada"
Medium Green	"Wilkin"
Dark Green	"Swift"

LEAF SIZE: The following varieties may be used as a guide to identify the relative size leaves.

SIZE	VARIETY
Small	"Amsoy"
Medium	"Bonus"
Large	"Anoka"

PLANT TYPE: The following varieties may be used as a guide to identify the plant type.

TYPE	VARIETY
Slender	"Vansoy"
Intermediate	"Wirth"
Bushy	"Adelphia"





Asgrow Seed Company
subsidiary of The Upjohn Company

Kalamazoo, Michigan 49001

1 July 1976

EXHIBIT D

Mr. Robert J. Snyder, Examiner
Plant Variety Protection Office
U.S. Department of Agriculture
National Agricultural Library, Rm. 301
Beltsville, Maryland 20705

Dear Bob:

Attached is data concerning additional replicated hypocotyl elongation tests comparing soybeans A3001 and Calland. These tests are in addition to those submitted May 18, 1976.

Please contact me should you have need of further information.

Very truly yours,

John A. Batcha, Manager
Inventory and Distribution

JAB/ghs

Enclosure

EXHIBIT D

Proof of Novelty of A3001 Soybean

The commercial soybean varieties most similar to A3001 to our knowledge are Wayne and Calland. Comparative characteristics which make A3001 a different variety include but are not restricted to the following:

A3001 Compared to Wayne

Flower color - A3001 Purple

Wayne White

A3001 Compared to Calland

Hypocotyl Elongation Tests

<u>Number of Plants out of 25 to Emerge</u>		
	<u>A3001</u>	<u>Calland</u>
Rep I	0	21
II	0	16
III	0	20
IV	<u>0</u>	<u>18</u>
Total	0	75

Tests were conducted by Asgrow in germinator set at 25°C. Seeds of high quality seedlots (warm germination tests of 90%+) were planted at a depth of 4 inches in sand:soil mixture (3:1). Emerged seedlings were counted 10 days after planting.

Tests were conducted April 2-12, 1976, at Ames, Iowa.

May 18, 197

Hypocotyl Elongation Tests - Conducted 6-4-76 - 6-14-76

In Germinator set at 25° C.
Each rep consisted of 25 seed planted at a 4" depth.
Three different seedlots were used for each variety.

No. of Plants at 10 days						
<u>Variety</u>	<u>Lot</u>	<u>REP</u>			<u>Total Out of 75</u>	<u>Rating</u>
		I	II	III		
A3001	A ¹⁾	0	0	1	1	5
	B ²⁾	0	0	0	0	5
	C ³⁾	0	2	1	3	5
Calland	A ⁴⁾	21	24	23	68	1
	B ⁵⁾	20	19	22	61	1
	C ⁶⁾	25	24	23	72	1

- 1) A3001 Lot A - Grown at Ames, Iowa, 1975
- 2) A3001 Lot B - Grown at Oxford, Indiana, 1975
- 3) A3001 Lot C - From Commercial Lot Grown at Perry, Iowa, 1975
- 4) Calland Lot A - Grown at Ames, Iowa, 1975
- 5) Calland Lot B - Grown at Oxford, Indiana, 1975
- 6) Calland Lot C - Foundation Seed purchased from Iowa Crop Improvement

1 July 1976

Asgrow Seed Company
Soybean A3001

EXHIBIT E

Statement of the Basis of Applicant's Ownership

Asgrow Seed Company purchased all rights to the soybean variety A3001 from Mike
Brayton Seeds Inc. in agreements dated April 9, 1974 and March 16, 1976.

March 23, 1976

Methods for Evaluation of Soybean Hypocotyl Length¹J. S. Burris and W. R. Fehr²

ABSTRACT

We compared the effectiveness and efficiency of sand and paper-towel methods for the evaluation of hypocotyl length of soybean (*Glycine max* (L.) Merrill) genotypes at 25°C. Seeds planted in sand at 10 cm in containers covered with plastic bags had hypocotyl lengths that were reproducible and consistent. Sand methods made individual hypocotyl measurements difficult. When the seeds were planted on standard germination towels, the results equaled those in the 10-cm sand if moisture was maintained at an adequate level. To obtain an adequate moisture level, it was necessary to use a 30- × 30-cm towel, and the containers had to be covered. Orientation of the seed with the micropyle up on the paper towel prevented twisted hypocotyls which were difficult to measure. Seed treatment with a fungicide had no influence on hypocotyl length, but may be of value with diseased seed lots.

Additional index words: *Glycine max* (L.) Merrill, Seedling emergence, Sand method, Paper towel method.

GRABE and Metzger (1) reported a temperature-induced inhibition of hypocotyl elongation that influenced seedling emergence. They reported inhibition of hypocotyl elongation at 25°C for the cultivars 'Amsoy,' 'Ford,' 'Adams,' 'Lincoln,' 'Shelby,' and 'Clark' of soybeans, *Glycine max* (L.) Merr. Their data indicated that inhibition increased as depth of planting increased, suggestion a soil resistance effect, as well as a temperature response. Inhibition of hypocotyl elongation is of considerable agronomic importance, since it may lead to unsatisfactory stand establishment under certain cultural practices, as indicated in the field study conducted by Grabe and Metzger (1). Although the method for evaluating hypocotyl length as outlined by Grabe and Metzger is satisfactory, rapid measurement of hypocotyl length is difficult. A paper towel method used by Samimy³ appeared to be more suitable for hypocotyl length measurements.

We studied various growth media and methods to determine the most efficient method that would reproducibly describe hypocotyl length of a genotype at 25°C.

MATERIALS AND METHODS

The soybean cultivars, 'Hawkeye' (long hypocotyl), 'Lindarin' (intermediate hypocotyl), and Amsoy (short hypocotyl), were used in this study. Ten methods of evaluation were compared,

¹ Contribution of the Iowa Agricultural and Home Economics Experiment Station, Journal Paper No. J-6633, Ames, Iowa, 50010, and the Crops Research Division, U. S. Department of Agriculture, manuscript No. 639 of the U.S. Regional Soybean Laboratory. Received Aug. 24, 1970.

² Assistant Professor of Botany and Associate Professor of Agronomy and Collaborator, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Iowa State University, respectively.

³ C. Samimy. 1970. Physiological bases for the temperature-dependent short growth of hypocotyls in some varieties of soybean. Unpublished Ph.D. thesis, Iowa State University, Ames, Iowa.

⁴ Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U. S. Department of Agriculture and does not imply its approval to the exclusion of other products that also may be suitable.

with each method replicated three times. Seeds were planted as follows.

- 1) 2.5 cm deep in clean sterile sand in 12-cm plastic pots, covered, and sealed with a plastic bag.
- 2) 2.5 cm deep in clean sterile sand in 12-cm plastic pots.
- 3) 10 cm deep in clean sterile sand in 12-cm plastic pots, covered, and sealed with a plastic bag.
- 4) 10 cm deep in clean sterile sand in 12-cm plastic pots.
- 5) On a 30- × 30-cm paper towel, rolled, and placed in a 3-liter jar, covered, and sealed with a plastic bag.
- 6) On a 30- × 30-cm paper towel, rolled, and placed in a 3-liter jar, covered, and sealed with a plastic bag. Seeds were treated with Arasan⁴.
- 7) On a 30- × 30-cm paper towel, rolled, and placed in a 3-liter jar, covered, and sealed with a plastic bag. Seeds were oriented with the micropyle down.
- 8) On a 30- × 30-cm paper towel, rolled, and placed in a 1-liter jar, covered, and sealed with a plastic bag.
- 9) On a 15- × 30-cm paper towel, rolled, and placed in a 1-liter jar, covered, and sealed with a plastic bag.
- 10) On a 10- × 30-cm paper towel, rolled, and placed in a 1-liter jar, covered, and sealed with a plastic bag.

We used 12 untreated seeds, except for Method 6, in both the sand and paper-towel methods. A standard, nontoxic, germination toweling was used for all paper towel methods. The seeds for the paper towel method³ were placed on the towels, along the 30-cm edge (Fig. 1) with the micropyle up, except for Method 7, so the seedlings would have the proper orientation for emergence. The seeds were placed on the top of two sheets of toweling, 4 cm from the edge, and covered with a third sheet, then carefully rolled with the seeds on the inside of the roll. The rolled towels were placed in the appropriate jars with the seed end up. To maintain moisture in the towel, 100 ml of water were added to the bottom of the jar. All tests were grown in the dark, at 25 ± 1°C, and hypocotyl lengths were determined 11 days after planting.

RESULTS AND DISCUSSION

The study involved four dates of hypocotyl evaluation. There were significant differences among test

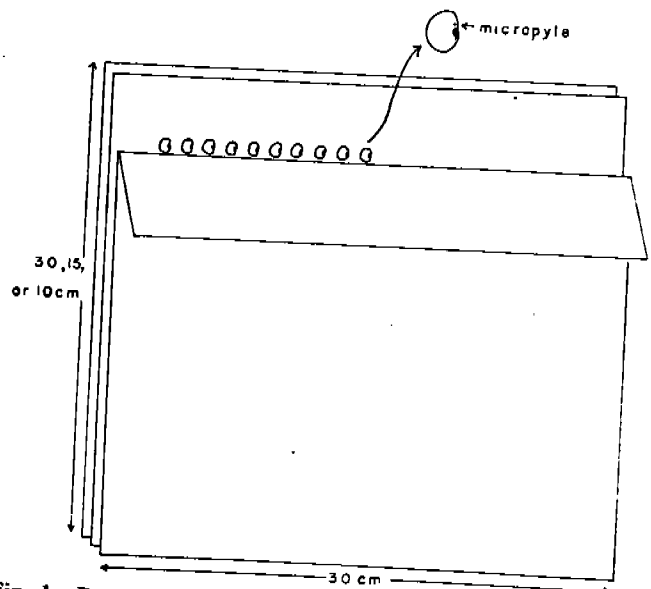


Fig. 1. Paper towel method. Three sheets of toweling were used for each test. A flap was made by folding down 3 cm of the top sheet. The seeds were planted, and the flap was placed back over the seed before the toweling was rolled.

dates, indicating the need for check cultivars, if genotypes grown at different times or in different controlled-environment facilities are to be compared directly. The lack of a significant test date \times cultivar interaction supported the validity of the comparison of hypocotyl lengths, adjusted to a check cultivar performance, across dates. Significant differences among methods and a significant methods \times cultivars interaction emphasized the difficulty in directly comparing hypocotyl length of genotypes evaluated by different methods.

All the methods tested satisfactorily separated the long and short hypocotyl class (Table 1). Differentiation of the intermediate class was best accomplished with the sand methods, except for Method 2.

The sand methods were satisfactory for evaluation of hypocotyl length when the moisture level of the sand was properly maintained. With 2.5 cm of sand, uncovered, there was difficulty in maintaining a proper moisture level. Adequate moisture is more easily maintained in plastic pots than in porous clay pots. Covering and sealing the pots with a plastic bag reduced drying and allowed better temperature control because of reduced evaporative cooling. The labor associated with covering the pots can be eliminated by planting in 10 cm of sand; however, certain types of sand form a hard surface crust upon drying and should be lightly watered to prevent crusting.

The sand method can be utilized in benches rather than pots if a temperature-controlled environment is available. A simple emergence rating can facilitate rapid evaluation, and planting depth can be varied to permit selection of any hypocotyl length desired. Where direct measurements of hypocotyl length are desired, the sand methods are not as efficient or convenient as the paper towel method.

Successful use of the paper towel method is dependent upon moisture content of the towel at seed level. A 35-cm long \times 73-cm wide towel was unsatisfactory because of excessive moisture around the seeds at the center of the roll. Reducing the width of the towel to 30 cm alleviated this problem. When different lengths of towel were compared (Table 1), we found that as the towel length was decreased from 30 to 10 cm, the reproducibility of the results decreased. Shorter towels had more seed rotting and abnormal seedlings, both of which were attributed to excessive moisture at seed level.

There was some concern that microbial growth was affecting the results. Based on a visual rating, treatment of the seed with a fungicide before planting did not reduce microbial growth. Treatment may be beneficial if the seed lots to be tested have a large degree of surface contamination. The use of a fungicide treatment did not influence hypocotyl length of the seedlings (Table 1).

Orientation of the seed as indicated in Fig. 1 is a useful step in the paper-towel method. Although the seed grows about as well when planted with the micro-

Table 1. Mean hypocotyl length (mm) of three soybean cultivars grown at 25C.

Method	Cultivars			SE*
	Amacy	Lindarin	Hawkeye	
	mm			
1) 2.5 cm sand, covered	82	149	172	5
2) 2.5 cm sand, uncovered	87	127	146	4
3) 10 cm sand, covered	87	110	165	6
4) 10 cm sand, uncovered	83	122	168	6
5) 30 \times 30-cm paper towel, 3-liter jar	95	132	146	5
6) 30 \times 30-cm paper towel, 3-liter jar, treated seed	93	140	152	4
7) 30 \times 30-cm paper towel, 3-liter jar, seed micropyle down	91	131	151	9
8) 30 \times 30-cm paper towel, 1-liter jar	84	130	133	9
9) 15 \times 30-cm paper towel, 1-liter jar	89	175	168	9
10) 10 \times 30-cm paper towel, 1-liter jar	113	167	181	9

* Standard error of the mean.

pyle up or down, the hypocotyl is more difficult to measure when the micropyle is down because of twisting from geotropism (Table 1). Covering the jars was necessary because of excessive drying of towels when they were left uncovered.

We compare 1- and 3-liter jars to determine if inhibition of hypocotyl elongation would be more severe in the smaller container. Kang et al. (2) and Samimy³ reported that reduced hypocotyl length may be due to ethylene produced by the soybean seedling. The volume of containers we used, however, did not affect hypocotyl length.

We evaluated the effect of short and long-hypocotyl cultivars on each other when grown in separate towels in the same 1-liter jar. Hypocotyl lengths of the cultivars 'Wayne' (long hypocotyl) and 'Cutler' (short hypocotyl) when grown alone or together were not significantly different. The results indicate several genotypes can be tested in the same jar without biasing the expression of hypocotyl length.

Since hypocotyl elongation is exponential with time, the time of measurement is critical. We have found that at 11 days after planting at 25C, plants of both the long- and short-hypocotyl cultivars have reached nearly their maximum length. Long- and short-hypocotyl cultivars are indistinguishable up to 7 days after planting, and growth measurements made earlier than 9 days may not permit separation of long and intermediate types.

The sand methods (Methods 3 and 4) gave the greatest differences between long, intermediate, and short hypocotyl genotypes. However, we believe the paper towel method (Method 5) to be the most efficient where large numbers of samples are involved and individual hypocotyl measurements are desired.

LITERATURE CITED

1. Grabe, D. F., and R. B. Metzger. 1969. Temperature-induced inhibition of soybean hypocotyl elongation and seedling emergence. *Crop Sci.* 9:331-333.
2. Kang, B. G., S. P. Burg, and P. M. Ray. 1967. Ethylene and carbon dioxide: Mediation of hypocotyl hook-opening response. *Science* 156:958-959.

Temperature-Induced Inhibition of Soybean Hypocotyl Elongation and Seedling Emergence¹

Don F. Grabe and Robert B. Metzger²

ABSTRACT

The effect of temperature on hypocotyl elongation and seedling emergence of soybean [*Glycine max* (L.) Merr] varieties was studied under laboratory and field conditions. Hypocotyl elongation of darkgrown 'Ford' seedlings was severely inhibited at 25 C, but was normal at 15, 20, and 30 C. Hypocotyl elongation of 'Hawkeye' was normal at all four temperatures. Twenty-five varieties were classified according to their ability to emerge from 10 cm of sand at 25 C. Distinct varietal differences in emergence ability were evident and appeared to be genetically controlled. Instances of erratic emergence of soybean plantings may be partially explained on the basis of depth of planting, variety, and soil temperatures during the germination period.

Additional index words: Field emergence, Stand establishment.

THIS study on the effect of depth of planting and temperature on emergence of soybean [*Glycine max* (L.) Merr] varieties was prompted by observations of erratic seedling emergence in the field. Erratic stands sometimes result when farmers increase planting depths to place the soybean seed in moist soil. Poor stands also have been frequently associated with certain varieties. Potential varietal differences in emergence ability were also suggested by differential seedling growth rates obtained in laboratory studies of seedling vigor. Information on emergence characteristics of soybean seedlings is pertinent to (a) making recommendations regarding planting depths and dates, (b) identifying breeding stocks that carry the poor emergence character, and (c) selection against poor emergence characteristics in development of future varieties.

MATERIALS AND METHODS

Seed lots

Seed of 'Chippewa,' 'Ford,' and 'Hawkeye' for use in temperature and depth studies was obtained from samples on file at the Iowa State University Seed Laboratory. These lots were produced at various locations in Iowa in 1965.

Seed of 25 varieties for studying varietal emergence characteristics was produced by C. R. Weber in varietal demonstration plots at the Iowa State University Agronomy Farm, Ames, in 1965. All seed was of good physiological quality and authenticated for varietal purity.

Laboratory experiments

Laboratory emergence studies were conducted in a substratum of concrete sand in clay pots or sand benches. Sand was steam sterilized before use.

The percentage emergence of Hawkeye and Ford soybeans from depths of 5, 7.5, and 10 cm was determined at 15, 20, 25,

and 30 C. For each treatment 25 seeds of each variety were planted in 20-cm diameter clay pots, with two replications.

Hypocotyl elongation in darkness was determined at temperatures of 15, 20, 25, and 30 C. Ten seeds each of Hawkeye and Ford were planted 2.5 cm deep in the same 20-cm clay pot, with two replications. A length of 20-cm diameter furnace pipe was placed over each pot to exclude light. The hypocotyls were measured after 21 days.

The rate of seedling emergence of three lots each of Hawkeye, Ford, and Chippewa was determined at temperatures of 20 and 25 C. Twenty-five seeds of each lot were planted 10 cm deep in 20-cm clay pots, with two replications of each treatment. Seedling counts were made daily until emergence was complete. Average laboratory germination for the three lots of each variety was 97, 96, and 97% for Hawkeye, Ford, and Chippewa, respectively.

Emergence of 25 varieties from depths of 5, 7.5, and 10 cm was determined by planting 50 seeds of each variety in rows in a sand bench, with three replications per variety. Sand temperature was 25 C.

Field experiments

Field emergence trials with Hawkeye and Ford were conducted in the spring of 1966 at Ames, Iowa. Planting depths were 4, 7.5, and 11.5 cm. Dates of planting were April 26 and May 25. Three replicates of 100 seeds each were planted.

RESULTS AND DISCUSSION

Hawkeye and Ford varieties differed markedly in their ability to emerge from deep planting in sand (Fig. 1). As shown in Table 1, Hawkeye emergence was similar at all temperatures and depths; emergence of Ford, however, was temperature dependent. At the 10-cm depth, maximum emergence of Ford was at-



Fig. 1. Seedlings of Hawkeye and Ford soybeans 15 days after planting 10 cm deep in sand at 20 and 25 C. From left to right: Hawkeye, 20 C; Ford, 20 C; Hawkeye, 25 C; Ford, 25 C.

Table 1. Percent emergence of Ford and Hawkeye soybeans from sand at three planting depths and four temperatures.

Variety	15	Temperature, C		
		20	25	30
		Planted 10 cm deep		
Hawkeye	96	94	98	98
Ford	86	62	4	74
		Planted 7.5 cm deep		
Hawkeye	100	96	94	100
Ford	80	82	56	78
		Planted 5 cm deep		
Hawkeye	100	98	96	98
Ford	86	92	88	90

* Laboratory germination: Hawkeye = 96% Ford = 84%.

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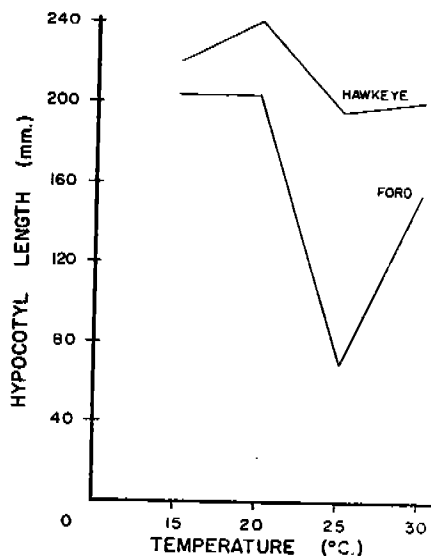


Fig. 2. Hypocotyl length of dark-grown Ford and Hawkeye soybeans 21 days after planting at four temperatures.

tained at 15 C, with only 4% emergence at 25 C. At 7.5 cm, lowest emergence was also attained at 25 C, while at 5 cm, emergence was complete at all four temperatures.

At 25 C, the germination percentage of Ford seeds was the same at all planting depths. At the 10-cm depth, however, the hypocotyls did not grow long enough to raise the cotyledons above the surface of the sand and emergence did not occur. Occasionally the epicotyl of a seedling emerged while the cotyledons remained below the surface, giving the appearance of a hypogeous seedling. The hypocotyls were frequently thickened and brittle, often breaking at the arch below the cotyledons. Conversely, deep planted Hawkeye seedlings were morphologically sound.

The morphological abnormalities of Ford seedlings suggested that failure of Ford to emerge from deep planting could be due to the weight and mechanical restriction of 10 cm of sand, together with a structurally weak hypocotyl. This hypothesis was tested by growing shallow-planted Ford and Hawkeye seedlings in the dark so the hypocotyls could develop normally in the absence of mechanical pressures from deep planting.

As shown in Fig. 2, hypocotyl growth of Ford was strongly suppressed at 25 C. The average hypocotyl length after 3 weeks growth was 70 mm, which would be insufficient to allow the seedlings to emerge from a depth of 10 cm. These results show that the inhibition of hypocotyl elongation from planting depths of 10 cm is primarily due to physiological causes, while the morphological abnormalities in deep planted Ford seedlings appear to be only secondarily related to failure of emergence.

Ford hypocotyl growth at 30 C showed a surprising increase over that at 25 C. This temperature response curve for elongation of dark-grown Ford hypocotyls is strikingly similar to the curve obtained by Went³ for germination of *Brassica arvensis* seeds. In that study, germination was slow but complete at cool tem-

³ Went, F. W. 1957. Experimental control of plant growth. *Chronica Botanica*, Waltham, Mass. 343 pp.

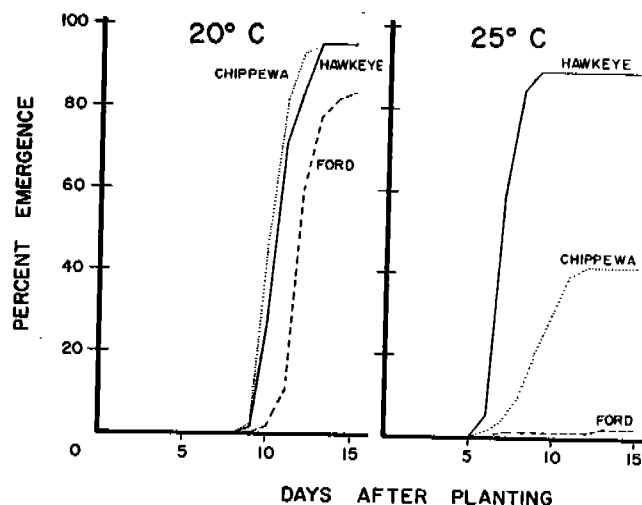


Fig. 3. Rate of seedling emergence of three soybean varieties when planted 10 cm deep in sand at 20 and 25 C. Seed viability: Hawkeye = 96%, Ford = 84%.

Table 2. Percent seedling emergence of 25 soybean varieties when planted at depths of 5, 7.5, and 10 cm in sand at 25 C.

Variety	Depth of planting, cm			Variety	Depth of planting, cm		
	5	7.5	10		5	7.5	10
Group 1				Group 2			
Hawkeye	99	100	100	Traverse	95	91	67
Dunfield	91	94	100	Lindarin	97	97	63
Mandarin 507	96	98	99	Illini	93	97	47
Blackhawk	98	97	96	Renville	97	76	46
Richland	98	99	94	Chippewa	96	84	23
E. M. Manchu	97	98	94	Group 3			
B. H. Manchu	93	98	93	Adams	93	75	13
Grant	94	98	92	Amsoy	91	69	6
Mukden	99	98	90	Lincoln	97	25	2
Mandarin (Ott.)	94	89	90	Shelby	96	16	1
Wayne	84	89	88	Clark	79	8	0
Seneca	86	87	86	Ford	88	2	0
Wisc. Manchu 606	91	76	83				
Harosoy	86	99	81				

Maximum range needed for significance at the 10 cm depth = 9.7 (Duncan's multiple range, 1% level).

peratures, almost completely inhibited at 26 C, but rapid and complete at 30 C. Went speculated that at 26 C inhibitors formed that almost completely suppressed germination, while at 30 C germination was faster than the rate of inhibitor formation. Such an inhibitor mechanism might be responsible for temperature controlled elongation of the Ford hypocotyl.

The seedling emergence rates of three seed lots each of Hawkeye, Ford, and Chippewa were studied to confirm that the emergence patterns found in the first experiment were due to varietal characteristics rather than physiological quality. There was no evidence of differences in emergence characteristics of lots within a variety. Mean values for emergence rates and total emergence of three lots of Ford, Chippewa, and Hawkeye from 10 cm at 25 C are therefore shown in Fig. 3. At 20 C, the emergence rates of all three varieties were similar. At 25 C, however, Hawkeye emergence was rapid and total, Ford emergence was nil, while Chippewa emergence was intermediate.

Twenty-five varieties were classified as to ability to emerge from sand at 25 C. This collection of varieties consisted of 11 common midwestern varieties together with their parental lines. Three groups were established on the basis of emergence at the 7.5 and 10-cm planting depths (Table 2). At the 10-cm depth, emergence of varieties in Group I was significantly

Table 3. Emergence characteristics of soybean varieties and parental lines. Symbols indicate emergence ability from sand when planted 10 cm deep at 25 C: * = poor; † = intermediate; ‡ = excellent.

Variety	Parentage
Amsoy*	Adams* × Harosoy†
Blackhawk†	Richland† × Mukden†
Chippewa†	Lincoln* × (Lincoln* × Richland)‡
Clark*	Lincoln* × (Lincoln* × Richland)‡
Ford*	Lincoln* × (Lincoln* × Richland)‡
Grant†	Lincoln* × (Lincoln* × Richland)‡
Hawkeye†	Seneca† × Lincoln*
Lindarint†	Richland† × Mukden†
Renville†	Mandarint (Ott.)† × Lincoln*
Shelby*	Lincoln* × (Lincoln* × Richland)‡
Wayne†	Lincoln* × (Lincoln* × Richland)‡
	CNS† × Lincoln* × (Lincoln* × Richland)‡

Table 4. Percent field emergence of Ford and Hawkeye soybeans from three planting depths at early and late planting dates.

Variety	Planted 11.5 cm deep		Planted 7.5 cm deep		Planted 4 cm deep	
	Apr. 26	May 25	Apr. 26	May 25	Apr. 26	May 25
Hawkeye*	72.0	64.0	87.3	78.7	97.3	93.0
Ford*	32.7	12.7	64.3	40.3	83.3	68.3

* Laboratory germination of Hawkeye = 96%, Ford = 84%.

greater at the 1% level of probability than varieties in Group II, and emergence of varieties in Group II was significantly greater than varieties in Group III, according to Duncan's Multiple Range Test⁴. Group I exhibited excellent emergence ability from all three depths. Group II emerged well from 5 and 7.5 cm, but emergence was considerably lower from 10 cm.

⁴ Duncan, D. B. 1955. Multiple range and multiple F tests. *Biometrics* 11:1-42.

Agronomic and Quality Characteristics of Awned and Awnleted Populations of Spring Wheat¹

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ABSTRACT

Awned and awnleted populations of spring wheat, *Triticum aestivum* L. em. Thell., derived by compositing seed from F₂ plants and F₃ lines classified as homozygous awned and homozygous awnleted, were compared for agronomic and quality performance at two locations. The awned population had heavier kernels and test weight at both locations and a 7% higher yield at one location. The awnleted population headed earlier and had more culms and more spikelets per head. Quality data showed the awnleted population superior in flour yield, loaf volume, farinograph stability, and farinograph viscometer.

The value of closely related populations for evaluating the effect of simply inherited characters is discussed.

Additional index words: Plants, Cereal, Culms, Spikelets, Farinograph, Flour.

THE productive advantage of the cereal awn has been investigated and evaluated for more than 30 years (1). Patterson et al. (10) have reviewed much of the literature on productivity of the awn, and they indicate there is generally an advantage to the awned type for test weight and kernel weight. A yield advantage has been shown for the awned types in some but not all materials tested. Evidence for awn superiority points to increased photosynthetic and physiological activity of the awn itself (8).

Group III showed considerably lower emergence from 7.5 and practically no emergence from 10 cm.

The inability of some varieties to emerge from 10-cm depths at 25 C because of insufficient hypocotyl elongation appears to be genetically controlled. As shown in Table 3, poor emerging varieties are from crosses involving poor emerging parents, but not all crosses involving poor emergers resulted in poor emerging offspring.

Five of the varieties studied trace to the 'Lincoln' × (Lincoln × 'Richland') cross. Three of these, 'Clark' Ford and 'Shelby,' are poor emergers. The other two, Chippewa and 'Renville,' are intermediate in emerging ability. This suggests that more than one gene is involved in the expression of the emergence character.

Field emergence data for Ford and Hawkeye (Table 4) followed the pattern expected on the basis of laboratory data. The percentage emergence of both varieties decreased with increased planting depth. The emergence of Ford, however, was reduced to a much greater degree with deep planting than was Hawkeye. Deep planting had a greater depressing effect on Ford at the later date of planting, presumably because of the increased soil temperatures.

The evidence obtained suggests that instances of erratic emergence of soybean plantings may be partially explained on the basis of depth of planting, variety, and soil temperatures occurring during the germination period. Physiological and genetic studies on elongation of the soybean hypocotyl are continuing.

Atkins and Finney (3) observed no quality differences but Lofgren et al. (9) obtained a higher flour yield from awnleted than awned lines. Atkins and Mangelsdorf (4) were first to suggest the use of isogenic lines for studying the influence of awns.

Recent studies comparing awned with awnleted or awnless wheat, *Triticum aestivum* L. em. Thell., and barley, *Hordeum vulgare* L. em. Lam., have been made with isogenic lines developed through a series of backcrosses (10, 11, 12). Isogenic lines have also been developed by selecting in successive generations for plants heterozygous for a given character, then selfing to fix the genotypes of the lines. This method of study has given valuable information, but development of the isogenic lines is tedious and time consuming.

A comparison of homozygous awned with homozygous awnleted plants from a segregating F₂ population

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